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(SYNOPSIS FOR REGISTRATION FOR DEGREE OF DOCTOR OF PHILOSPHY IN BOTANY)

Proposed Topic	:	Pollination, pollen germination and course of pollen tube growth in some crucifers.
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1. Background:

1. a The process of fertilization in angiosperms is preceded by pollination whereby pollen grains are carried from the microsporangium to the pistil. This process is achieved through the intervention of various biotic and abiotic agents. Pollination has been necessitated by the closure of carpel and embedding of the megasporangium and the female gametophyte contained therein deep within the ovarian tissue. A few exceptions apart, angiosperm pistil has three distinct parts, the stigma, style and the ovary, each specialized to discharge specific role to facilitate fertilization.

Stigma is the receptive part of pistil where pollen grains are deposited by the pollinators. Although, it receives pollen grains of all kinds it is equipped with the ability to sieve them, reject the alien and allow only the right pollen to germinate. This is the first phase in the long and complex process of pollen - pistil interaction (Bhojwani and Bhatnagar, 1974; Raghvan, 2000). The compatible pollen is recognized through the interaction between the leacheates of the pollen wall and the substances contained in the stigmatic exudates. Once accepted, the pollen grains germinate and produce one or more pollen tubes which may be branched or unbranched. (Maheshwari, 1950; Bhojwani and Bhatnagar, 1974; Shivanna, 1982; Johri, 1984).

The pollen tubes make their way through the stigma into the style which is hollow in some and semi - solid or solid in other angiosperms (Bhojwani and Bhatnagar, 1974). The pollen tube and the stylar tissue are also involved in a recognition process. Only if there is perfect compatibility between the two, do the pollen tubes continue their journey towards the ovary. In event of incompatibility between the two the pollen tubes either stop growing at stigmatic surface or burst within the style.

The compatible pollen tubes enter the ovule through poro, meso or chalazogamy. They make way through the nucellar tissue into the embryo sac and enter it through one of the two synergids. In only such embryo sacs which lack synergids does the pollen tube enter the egg cell directly (Raghvan, 2000).

Pollen tubes discharge their contents, comprising the two sperms and the vegetative cell nucleus into the synergid or the egg cell. Finally, one of the sperms fuses with the egg nucleus and

the other with the secondary nucleus. Thus are formed the zygote and the primary endosperm nucleus.

1.b Anomalies in the seat of pollen germination:

While fertilization of vast majority of angiosperms conforms to the above description many taxa exhibit differences. One of the anomalies reported concerns the seat of pollen germination. In some plants pollen grains germinate at places other than the stigma. In the tiny cliestogamous flowers of the species of four genera, namely *Aspicarpa, Camarea, Janusia* and *Gaudichaudia* of family Malpighiaceae pollen grains are known to germinate inside the indehiscent anthers (Anderson, 1984). In *Trifolium dubium* also the pollen grains germinate inside the anther and the germinating pollen grains with their pollen tubes intact fall on the stigma surface and cause fertilization (Sharma and Koul, 1996). Swamy (1974) observed precocious pollen germination inside the anthers in *Gnetum ula.* Pollen grains are also known to germinate on the moist suface of extra floral parts, particularly the petals (Juliano, 1935; Lee *et al*., 1978) and nectary (Parihar *et al*., 2009). In several members of families Brassicaceae, Capparidaceae and Papaveraceae pollen grains are reported to germinate on ovary wall besides the stigma (Sharma *et al*., 2001, Sharma, 2002 and Sharma, unpublished data).These observations were, for long, dismissed as freaks and aberrations which fortified the belief that all flowering plants conform to a single mode of pollen tube growth from stigma to the embryo sac as outlined above.

1.c Anomalies in pollen tube growth:

Two papers, published in 1980's brought to light sound evidence that in some plants pollen tubes adopt courses other than the style for their journey from the stigma to the ovary (Anderson, 1984 and Philbrick, 1984). Species of *Callitriche* and some members of family Malphigiaceae were considered apomicts for long because no body had ever noticed their anthers dehisce and shed pollen. Nor was the pollen ever seen germinating on their stigma. Yet, they bore abundant seed.

In species of *Callitriche* the flowers are unisexual but both types are borne on the same plant. Philbrick (1984) observed that in *Callitriche heterophylla* and *C. palustris* the pollen grains germinate inside anthers and pollen tubes produced therefrom make way through the filament of the stamen into the thalamus from where they enter the pistil and finally reach the embryo sac and effect fertilization.

In *Sagittaria potamogettifolia*, a member of family Alismatacae, when more than one pollen grain are deposited on a stigma, all of them germinate and produce pollen tubes which travel down the style and reach the basal part of ovary. When one of these pollen tubes enters the ovule through its micropyle, the rest of pollen tubes continue growing and reach the receptacle.Thereafter, they change their course to reach the basal part of the neighbouring pistil. Finally, they enter the ovule and effect fertilization (Wang *et.al.*,2002).

2. Justification of the proposed work :

Following the above publications doubts started arising about acceptability of stigma as the only site of pollen germination and style as the only path of pollen tube journey from stigma to the ovary. These doubts motivated studies which led to some more interesting observations. Sharma *et al.*, (2001) discovered that in *Thalaspi arvense* and many other crucifers pollen grains germinate at two sites; stigma surface and the ovary wall. Pollen tubes formed at the stigma adopt the usual course of travelling through the stylar tissue to reach the ovary. The pollen tubes emerging from pollen grains at the ovary wall enter the pistil through the numerous stomata dispersed all over the ovary wall. Once inside the ovary they enter the ovule and fertilize the egg.

Recently, Parihar *et al.*, (2009) made similar observations in *Artemisia maritima*, a member of family Asteraceae, which is distinct from families of the order Parietales, where it had been reported earlier. In *A. maritima* pollen grains germinate at the nectary and produce pollen tubes which enter the ovary directly and effect fertilization.

Having discovered the novel sites of pollen grain germination and novel course of pollen tube growth neither those who discovered these novelities nor any one thereafter has attempted to

evaluate the efficiency of these processes and compare them with the standard site of pollen germination and pollen tube growth.

The proposed investigation aims at comparing the two modes of pollen grain germination and pollen tube growth in some such members of Brassicaceae which practice the dual modes hand - in - hand and by so doing answer the plethora of questions that observations on *Thalaspi arvense* and *Artemisia maritima* have raised.

3. Objectives:

The specific objectives of the proposed work are:

To,

(i) survey as many members of family *Brassicaceae* as are locally available for determining the pollination mechanism ,pollen germination and pollen tube growth.

(ii) determine extra stigmatic sites for pollen germination;

(iii) compare chemical composition of the exudates that trigger pollen germination at the stigma surface and ovary wall;

(iv) compare the time taken by pollen to germinate on the surface of stigma and the surface of ovary wall following controlled pollination;

(v) compare the taxa that practice ovarian pollination with their allies that do not with regard to presence, absence and frequency of stomata on the ovary wall;

(vi) compare rate of growth of pollen tubes that emerge at stigma and the ovary wall;

(vii) collect evidences, direct and indirect, to prove unambiguously that pollen tubes emerging at the ovary wall cause fertilization, and

(viii) determine the effect, if any, on seed and fruit set by inhibiting pollen germination on stigma and vice- versa.

4. Materials proposed to be used for the investigation:

(i) For Objectives (i & ii) attempt will be made to explore as many taxa of family *Brassicaceae* as are available at Rajouri and its immediate neighbourhood.

(ii) For objectives (iii) - (viii) a minimum of 4 wild/ cultivated taxa will be selected, which exhibit duality in the site of pollen germination and path of pollen tube growth and entry into the ovary. Preference will be given to taxa with large flowers to facilitate manipulation.

5. Methodology proposed to be followed:

Following tools and techniques shall be used for the proposed investigation.

i) Floral morphology: Field surveys to collect locally available members of family *Brassicaceae*. Floral morphology of the selected taxa will be studied to study the floral structure.

ii) Floral-biology: Data will be collected on anthesis , anther-dehiscence and stigma receptivity using the techniques of Lewis (1979) and Shivanna and Rangaswamy (1992).

iii) Pollination mechanism, mode of pollination will be ascertained through visual observations, bagging experiments and hanging slide technique. The plant will be checked for nature of attractants and rewards offered by their flowers. Their influence on pollinator activity will be determined by field studies and experimentation.

iv) Exudates of the stigma and ovary wall (if any) will be analysed and compared for composition using specific staining techniques namely Coomassie brilliant blue and Sudan black.

v) Efficiency of pollen germination on ovary wall will be determined by excising the stigma and style and covering the cut-end with petroleum jelly and bagging the pollinated ovaries.

vi) Pollen tube growth rate will be determined by Aniline Blue-fluorescence method. The stigmas and ovary will be pollinated periodically, excised at regular intervals and fixed in a mixture of 3 parts absolute alcohol and 1 part acetic acid for 4-6 hours and cleared in 1N NaOH in an electric oven maintained at 60°C for 1-2 hours. Thereafter, the stigmas will be washed with distilled water and mounted in 1:1 V/V mixture of decolorized 0.005% Analin Blue and 50% glycerin.

vii) Ovary wall will be checked for stomata, by peeling off its epidermis, staining it with Fast Green and mounting it in water or 30% Safranin. Stomatal frequency will be determined by following formula;

Area of the peel = X^2 mm

No. of stomata = Y

Ferquency of stomata at X^2 area= Y/X^2

Total No. of stomata on the ovary wall = Total Area of the ovary wall x No. of stomata present in X^2 area .

viii) Controlled pollination will be undertaken by clipping off the stigma of a pistil and dusting the ovary wall with pollen and bagging the flowers till they set seed. Bags will be removed only when the fruit and seed set data are to be recorded.

viii) The last objective will be achieved by first excising the stigma while still non receptive, sealing the cut end of style with paraffin wax, permitting pollen to germinate on ovary wall and estimating the seed and fruit set. The pollen tube path will be determined by fluorescence microscopy.

6. Possible outcome of the investigation;

After the investigation is completed answers should become available to the question, whether pollen germination on ovary wall, results in fertilization, seed set and fruit set or not. If it does how does it compare with stigmatic pollination in efficiency.

Bibliography:

1. Anderson, W. R. 1984. Cryptic self fertilization in the Malpighiaceae. Sci. **207** : 892-893.
2. Bhojwani, S. S and Bhatnagar, S. P. 1974. Embryology of Angiosperms-Vikas Publishing House, Pvt.Ltd. New Delhi.
3. Johri, B. M. 1984. Embryology of Angiosperms. Springer - Verlag, Berlin.
4. Juliano, J. B. 1935. Anatomy and morphology of the Bunga, *Aeginetia indica* L. Philippne J. Sci. **56** : 405 – 451.
5. Lee, C.W., Erickson, H.T. and Janik, J. 1978. Chasmogamous and cleistogamous pollination in Salpiglossis sinuate.Physio. Plant. **43** : 225-230.
6. Maheshwari, P. 1950. An Introduction to the Embryology of Angiosperms. Mc Graw. Hills Book Co. Inc., New York.

7. Philbrick, C. T.1984. Pollen tube growth within tissue of *Callitriche*(*Callitrichaceae*). Am. J. Bot. **71** : 882-886.

8. Philbrick, C.T. 1992. Pollen biology in Callitriche (*Callitrichaceae*). Systematic Bot. **17** : 282-292.

9. Parihar, J., Hamal, I. A. and Sharma, N. 2009. Dual pollen germination in *Artemisia maritima* L. Curr. Sci. **97** : 24-25.

10. Raghvan, V. 2000. Developmental Biology of Flowering Plants. Springer -Verlag, New York.

11. Sharma, S. 2002. Studies on Reproductive Biology and Ex-situ Conservation of Some Threatened Plants of North-West Himalya, Ph.D thesis approved by University of Jammu.

12. Sharma, N. and Koul, A. K. 1996. In situ pollen germination and selective ovule abortion in *Trifolium dubium* Sibth. Curr. Sci. **71** : 598.

13. Sharma, S., Kaul, V., Magotra, R. and Koul, A. K. 2001. Pollen germination on ovary wall and consequences thereof. Current Sci. **80** : 824-826.

14. Shivanna, K. R. and Rangaswamy, N. S. 1992. Pollen Biology - A Laboratory Manual. Narosa Publishing House, New Delhi.

15. Shivanna, K. R. 1982. "Pollen pistil interaction and control of fertilization," PP. 131-174; In Johri; B.M. (ed.), Experimental Embryology of Vascular Plants. Springer - Verlag, Berlin.

16. Wang, Xiao-Fan, Tao,You-Bau and Lu, Ying-Tang. 2002. Pollen tubes enter neighbouring ovules by way of receptacle tissue, resulting in increased fruit set in *Sagittaria potamogettifolia*, Ann. Bot. **89** : 791-796.